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Abstract: Genetic manipulation of the unicellular green alga Chlamydomonas reinhardtii is straightforward. Nuclear genes can be interrupted by insertional mutagenesis or targeted by RNA interference whereas random or site-directed mutagenesis allows the introduction of mutations in the mitochondrial genome. This, combined with a screen that easily allows discriminating respiratory-deficient mutants, makes Chlamydomonas a model system of choice to study mitochondria biology in photosynthetic organisms. Since the first description of Chlamydomonas respiratory-deficient mutants in 1977 by random mutagenesis, many other mutants affected in mitochondrial components have been characterized. These respiratory-deficient mutants increased our knowledge on function and assembly of the respiratory enzyme complexes. More recently some of these mutants allowed the study of mitochondrial gene expression processes poorly understood in Chlamydomonas. In this review, we update the data concerning the respiratory components with a special focus on the assembly factors identified on other organisms. In addition, we make an inventory of different mitochondrial respiratory mutants that are inactivated either on mitochondrial or nuclear genes.

Opposed Reviewers:

October 7th 2013

Dr Buckingham

Editor of Biochimie

Ms. Ref. No.: BIOCHI-D-13-00330
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Dear Dr. Buckingham,

Please find enclosed a revised version of our manuscript "Respiratory-deficient mutants of the unicellular green alga Chlamydomonas: a review." (BIOCHI-D-13-00330).

First, we wish to thank the two anonymous reviewers for their constructive comments. We found their suggestions very helpful to improve the quality of our manuscript. We have addressed all the points they raised and have modified the text accordingly. A detailed response to reviewers' comments is included below.

We believe we have addressed all referees' comments and we hope this revised version is now suitable for publication in Biochimie.

We are looking forward to hearing from you,

With best regards,

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Response to reviewers

Response to reviewer 1

1. *Overall, the review is fine, but a bit too descriptive and I feel that more background detail could be given for the general reader on the methodology of how to create respiratory mutants (explanation of transformation methods and selection strategies for creating nuclear and mitochondrial mutants. The TTC-based screen and dark-dier/slow growth screens for different classes of mutant, etc.).*

The text has been modified in order to give more background details on the methodology used to create and screen respiratory mutants in *Chlamydomonas*. The main modifications correspond to:
Paragraph 2.3:
- p8. A figure (Fig. 4) has been added in order to provide more information on dark-dier/slow growth screens for different classes of mutant.
- p9. The mitochondrial transformation method used in *Chlamydomonas* has been detailed.

Paragraph 2.4:
- p10. The TTC-based screen has been explained.

Finally, all along the text the methods used to create nuclear and mitochondrial mutants has been indicated.

2. *Also, the text could benefit from being proof-read by a native English speaker as the grammar is a little awkward in places. I highlight a couple of points below, but there are a number of other places where the English could be improved for clarity.*

The text has been proof-read by a good English speaker as requested and the text has been modified according to the comments.

Minor points:

1. Abstract, line 1: “the genetics” have been replaced by “genetic manipulation”

2. Abstract, line 1: “algae” have been replaced by “alga”

3. Page 6, line1: the sentence has been modified

4. Page 10, line 57: “that” have been deleted

5. Page 13, line 42: “mutant” have been deleted

Response to reviewer 4

1. *It would be visually interesting to add a diagram like a histogram showing the number of structural and assembly components for each complex in each organism*

p7. Figure 2 showing the number of structural and assembly components for each complex in each organism has been added.

2. *Cbp3 and Cbp6 are cited in Table 1 as assembly factors for complex III in yeast, however more precisely they are important for the stability of neo-synthetized cytochrome b as well as for optimal translation of the cytochrome b mRNA. Similarly Mss51, which is not cited in Table 1, is both a translation factor for the COX1 mRNA and an early assembly factor for the Cox1 protein, thus mediating a feedback regulation of COX1 translation. Since they have some post-transcriptional function but not only, the author should either cite all three factors in Table 1 or leave out all three, and explain in the legend what they decided to do for these dual function factors.*

In Table 1, we did not include the factors that are exclusively involved in the expression of the OXPHOS subunits. However, proteins as Cbp3, Cbp6 and Mss51 that have a post-transcriptional function but have also a respiratory complex assembly function are cited. As pointed out by the reviewer, the Mss51 was not cited in Table 1 and this omission has been fixed.

3. *Table 1 should be checked for errors inserted during the update of the 2012 paper. For example an alternative oxidase reference Q9Y711U is given for S. cerevisiae, which does not have any AOX. In the 2012 paper, the same reference is given as Q9Y711u , the u pointing to a note explaining that the sequence is from Pichia stipitis. In Pubmed, Q9Y711 actually refers to an Ajellomyces capsulatus AOX sequence. Please remove the U and cite the proper organism for this sequence.*

Table 1 has been proofed in order to avoid these errors.
Concerning the AOX protein of yeast, the protein reference has been changed and replaced by the reference of the AOX of *Neurospora crassa* (accession AAC37481).

Reference:
[79]: Q. Li, R.G. Ritzel, L.L. McLean, L. McIntosh, T. Ko, H. Bertrand, F.E. Nargang, Cloning and analysis of the alternative oxidase gene of *Neurospora crassa*, Genetics 142 (1996) 129-140.

4. *Page 6 lines 4-5, it is said that seven Chlamydomonas proteins are found in other eukaryotic complexes, where a seventh one encodes the homolog of COX5c. It would be "eighth" one and not "seventh" one, however I think that the confusion comes from the fact that Chlamydomonas COX2 is a split subunit, the N- and C-termini being encoded by two peptides. The authors should comment on that interesting feature and call the two COX2 proteins "a" and "b" in Table 1 (instead of COX2 and COX2a).*

p 6. In order to avoid the misleading, the particularity of the COX2 protein of *Chlamydomonas* has been commented in the text and the suggestion of the reviewer to call the two COX2 proteins “a” and “b” has been taken into account in Table 1.

5. *In Table 1, COX4/5b from Chlamydomonas (XP_001693699) is rather supposed to be the homolog of human COX5b and yeast COX4, it should be moved two lines up.*

The modification has been done in Table 1.

*Author Agreement

11 July 2013

Université de Liège

Dear Dr Buckingham,

By this letter, I confirm that all the authors of the manuscript submitted agree with its content.

Sincerely yours,

Corresponding author

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Abstract

Genetic manipulation of the unicellular green alga *Chlamydomonas reinhardtii* is straightforward. Nuclear genes can be interrupted by insertional mutagenesis or targeted by RNA interference whereas random or site-directed mutagenesis allows the introduction of mutations in the mitochondrial genome. This, combined with a screen that easily allows discriminating respiratory-deficient mutants, makes *Chlamydomonas* a model system of choice to study mitochondria biology in photosynthetic organisms. Since the first description of *Chlamydomonas* respiratory-deficient mutants in 1977 by random mutagenesis, many other mutants affected in mitochondrial components have been characterized. These respiratory-deficient mutants increased our knowledge on function and assembly of the respiratory enzyme complexes. More recently some of these mutants allowed the study of mitochondrial gene expression processes poorly understood in *Chlamydomonas*. In this review, we update the data concerning the respiratory components with a special focus on the assembly factors identified on other organisms. In addition, we make an inventory of different mitochondrial respiratory mutants that are inactivated either on mitochondrial or nuclear genes.

Respiratory-deficient mutants of *Chlamydomonas*: a review

Thalia Salinas, Véronique Larosa, Pierre Cardol, Laurence Maréchal-Drouard and Claire Remacle

Highlights

- *Chlamydomonas* respiratory-deficient mutants can be isolated.
- They are mutated in mitochondrial or nuclear genes.
- Random insertional mutagenesis and RNA interference can be used to target nuclear genes.
- Random and site-directed mutagenesis can be used to target mitochondrial genes.

Respiratory-deficient mutants of the unicellular green alga *Chlamydomonas*: a review

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1. The mitochondrial respiratory chain of *Chlamydomonas*

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1.2 Alternative enzymes

2. The respiratory-deficient mutants of *Chlamydomonas*

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2.6 Mutants affected in alternative enzymes

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3. Conclusion

Abstract

Genetic manipulation of the unicellular green alga *Chlamydomonas reinhardtii* is straightforward. Nuclear genes can be interrupted by insertional mutagenesis or targeted by RNA interference whereas random or site-directed mutagenesis allows the introduction of mutations in the mitochondrial genome. This, combined with a screen that easily allows discriminating respiratory-deficient mutants, makes *Chlamydomonas* a model system of choice to study mitochondria biology in photosynthetic organisms. Since the first description of *Chlamydomonas* respiratory-deficient mutants in 1977 by random mutagenesis, many other mutants affected in mitochondrial components have been characterized. These respiratory-deficient mutants increased our knowledge on function and assembly of the respiratory enzyme complexes. More recently some of these mutants allowed the study of mitochondrial gene expression processes poorly understood in *Chlamydomonas*. In this review, we update the data concerning the respiratory components with a special focus on the assembly factors identified on other organisms. In addition, we make an inventory of different mitochondrial respiratory mutants that are inactivated either on mitochondrial or nuclear genes.

Highlights

Chlamydomonas respiratory-deficient mutants can be isolated.

They are mutated in mitochondrial or nuclear genes.

Random insertional mutagenesis and RNA interference can be used to target nuclear genes.

Random and site-directed mutagenesis can be used to target mitochondrial genes.

Keywords (4)

Chlamydomonas, mitochondria, respiratory chain, respiratory-deficient mutants

1. The mitochondrial respiratory chain of *Chlamydomonas*

Mitochondria are the site of oxidative phosphorylation (OXPHOS). This process comprises an electron-transfer chain that is driven by substrate oxidation and is coupled to the synthesis of ATP through an electrochemical transmembrane gradient. Therefore, the mitochondrial respiratory-chain proteome comprises protein components participating in this process (complexes I-V and additional oxidoreductases) and in its biogenesis (assembly factors).

The release of the complete genome sequence of the unicellular green alga *Chlamydomonas reinhardtii* allowed the construction of a comprehensive catalog of its OXPHOS components [1]. Here, we present as an introduction an update of the inventory set up in that review, with special focus on assembly factors that have been identified in mammals or fungi since that time.

1.1 Main components of the respiratory chain

In **Table 1** we provide a summary of the components of the respiratory chain in different organisms.

Mitochondrial complex I (NADH:ubiquinone reductase, EC 1.6.5.3) is one of the three energy-transducing enzymes of the electron transfer chain in mitochondria. Complex I is the main entry point of the electrons in the respiratory chain and catalyzes NADH oxidation and ubiquinone reduction. Coupled to electron transfer, protons are pumped from the matrix side into the intermembrane space. With an L-shape structure and an apparent molecular mass of ca. 1000 kDa, it comprises 44-45 subunits in *Bos taurus* [2, 3]. In *Arabidopsis thaliana*, 49 distinct subunits have been identified [4], 40 of which are homologous to mammal subunits [5]. In *Chlamydomonas*, the subunit composition is almost similar to the one described in *A. thaliana* [5, 6]. Five (*e.g.*, in *Chlamydomonas*) to nine (*e.g.*, in land plants) subunits (the ND or NAD subunits) are encoded in the mitochondrial genome, whereas the remaining subunits are nuclear gene products. The 14 conserved core subunits, homologous to the bacterial type enzyme subunits are sufficient for energy transduction while the supernumerary proteins are not required for catalysis and the reason of their presence is still matter of debate. Some supernumerary proteins have independent roles (like the B16.6 subunit in apoptosis) but others have nonspecific

roles in regulation, protection against reactive oxygen species, assembly and stability [7]. In *Arabidopsis*, green algae and amoebozoa, subunits structurally related to gamma carbonic anhydrases have been found [6, 8, 9]. Single particle electron microscopy analyzes of complex I from *Polytomella* (a chloroplast-less and close relative of *Chlamydomonas*), *A. thaliana*, *Zea mays* and *Solanum tuberosum* indicate that these γ-CA subunits could constitute a spherical domain attached to the central part of the membrane arm of complex I, and exposed to the matrix (**Fig. 1A**) [10-13]. In addition, nine chaperones (or assembly factors) that participate to the biogenesis of complex I have been identified in human and fungi. The first two assembly factors, CIA30 and CIA84, identified in the fungus *Neurospora crassa*, participate to the assembly of the membrane domain [14]. Of these two chaperones, only CIA30 is conserved in mammals and plants. Another chaperone, IND1, is participating in the assembly of Fe–S cofactors and subunits of complex I in yeast *Yarrowia lipolytica* [15] and is well conserved in human [16] and plants. In the past few years, the discovery of six assembly factors (C20ORF7, C8ORF38, FOXRED1, NDUFAF2, NDUFAF3, and NDUFAF4) provided a significant insight into the assembly process of human complex I (see [17] for a review). All of them are conserved in *Chlamydomonas* except B17.2L, a paralog of the B17.2 subunit.

Complex II or succinate:ubiquinone oxidoreductase (EC 1.3.99.1) is an enzyme involved both in the Krebs cycle and the respiratory chain. Complex II is composed by four distinct nucleus-encoded polypeptides, SDH1 (the flavoprotein subunit), SDH2 (the iron-sulfur subunit), and two hydrophobic membrane anchors, SDH3 (subunit III) and SDH4 (subunit IV). The genes encoding the corresponding subunits are all present in *Chlamydomonas* [1]. Of the four assembly factors identified to date, three assembly factors have homologs in *Chlamydomonas*, an assembly factor (SDHAF1), a membrane transporter (FLX1) and a flavinylation factor (SDH5).

The 10 classical eukaryotic subunits of complex III (ubiquinol:cytochrome *c* oxidoreductase, EC 1.10.2.2) are found in the genome of *Chlamydomonas* [1]. Amongst them, cytochrome *b*, cytochrome *c1*, and the Rieske iron-sulfur (Fe/S) protein, which all exhibit high sequence similarity to their bacterial counterparts, are essential for the catalytic activity while the other proteins are the so-called supernumerary ones. On the four assembly factors that participate to the formation of the mature and functional complex III enzyme, three have putative homologs in

Chlamydomonas. Cytochrome *c* is nucleus-encoded and the type-III maturation system is used, like in mammal and fungi [1].

The *Chlamydomonas* genome encodes six proteins found in other eukaryotic complexes IV (EC 1.9.3.1), whereas a seventh one encodes the homolog of the plant-specific COX5c subunit [18]. A *Chlamydomonas*-specific protein possibly involved in complex IV assembly and considered to belong to the enzyme complex, Cox90, is also found [19]. The COX catalytic core is formed by three subunits, COX1, COX2 and COX3, conserved in the bacterial enzyme. In *Chlamydomonas*, only COX1 is encoded in the mitochondrial genome while Cox2 and Cox3 are nucleus-encoded [20, 21]. A peculiar fact of the *Chlamydomonas* Cox2 protein is that it is a split subunit, the N- and C-termini being encoded by two proteins (Cox2a and Cox2b). These two proteins assemble with other complex IV subunits to form the mature complex [20]. Numerous factors involved in processes related to complex IV such as membrane insertion and processing, copper metabolism and insertion or heme A biosynthesis have been identified. Most of them have putative homologs in *Chlamydomonas*.

At last, the first factor implicated in the assembly of supercomplexes III and IV was recently described in yeast and humans [22]. No homolog was found in plants and *Chlamydomonas*.

Complex V or F_1F_O-ATP synthase (EC 3.6.3.14) works as a rotary motor driven by an electrochemical proton gradient [23]. Proton translocation through the F_O sector drives rotation of the central stalk (gamma subunit) that extends from the membrane-embedded c-ring into the center of the F_1 sector. The conformational changes induced by rotation of gamma subunit in F_1 allow the synthesis of ATP in the catalytic sites of the beta subunits [24]. Like other chlorophycean algae, *C. reinhardtii* exhibits a highly stable dimeric mitochondrial F_1F_O-ATP synthase with an apparent molecular mass of 1600 kDa [25]. This dimeric enzyme complex has a unique architecture with a robust peripheral stalk [26-29] (**Fig. 1B**). The functional core of the algal enzyme is formed by eight classical subunits [α (encoded by *ATP1*), β (encoded by *ATP2*), γ (encoded by *ATP3*), δ (encoded by *ATP16*), ε (encoded *ATP15*), *a* (encoded by *ATP6*), *c* encoded (*ATP9*), and OSCP (encoded by *ATP5*)] and nine atypical subunits (encoded by the *ASA1-9* genes), exclusively present in the chlorophycean lineage, that constitute the robust peripheral stalk and seem to participate in the dimerization of the complex [25,

30, 31]. Factors involved in the F_O and F_1 assembly have been identified in *S. cerevisiae* and four of them have putative homologs in *Chlamydomonas*.

In **Fig. 2** are presented histograms showing the number of components for each complex and the corresponding assembly factors identified. Although the number of subunits is the highest for complex I, one can notice that the number of assembly factors is very low compared, for example, to what is known for complex IV.

1.2 Alternative enzymes

The *Chlamydomonas* nuclear genome encodes six type II NAD(P)H dehydrogenases (Nda1, 2, 3, 5, 6, 7), and the localization of three of them has been determined: Nda2 and Nda3, are located in the chloroplast [32, 33] while Nda1 is located at the inner side of the inner mitochondrial membrane [34].

In addition, two nuclear genes encoding alternative oxidase enzymes (Aox1 and Aox2) are found, the *AOX1* gene being much more transcribed than *AOX2* [35]. The *AOX1* expression is strongly dependent on the nitrogen source, being down regulated by ammonium and stimulated by nitrate [36].

2. The respiratory-deficient mutants of *Chlamydomonas*

2.1 Phenotype of respiratory-deficient mutants

The unicellular green alga *Chlamydomonas* can grow photoautrophically using CO_2, heterotrophically using acetate and mixotrophically using both carbon sources. Acetate is metabolized following its entry into the Krebs cycle, which feeds the respiratory chain with reducing equivalents. Therefore, *Chlamydomonas* respiratory-chain deficient mutants are easily identified by the null or slow growth in conditions where growth only relies on respiration, *e.g.* in the dark with acetate as carbon source (heterotrophic conditions).

2.2 Genetics of *Chlamydomonas* to isolate respiratory-deficient mutants

The first nuclear respiratory-deficient mutants were described in 1977 after mutagenic treatment with nitrosoguanidine by the group of Boynton and Gillham [37]. These mutants displayed slow or null growth in the dark (dk-dier or dk^-), they were defective for cytochrome *c* oxidase activity and exhibited altered mitochondrial structure. The growth phenotype was inherited in a Mendelian fashion demonstrating the nuclear location of the mutation. More recently, insertional mutagenesis (*e.g.*, [38]) or RNA interference technique (*e.g.*, [39], [40]) were used in order to target nuclear genes involved in respiration (see below for the description of the mutants).

The first mitochondrial mutants were isolated by random mutagenesis with the intercalating dyes acriflavine or ethidium bromide [41]. The null growth phenotype of these mutants under heterotrophic conditions was inherited by the mating type minus parent, a characteristic of a genetic lesion located in the mitochondrial genome [42]. The respiratory-deficient mutants affected in mitochondrial genes are thus called *dum*, standed for dark uniparental minus. More recently, mitochondrial transformation was set up with the aim of performing site-directed mutagenesis of the respiratory subunits encoded by the mitochondrial genome (*e.g.*, [43], [44]). Therefore *C. reinhardtii* is the only photosynthetic organism where reverse genetics is possible in mitochondria.

In **Table 2** are listed the different mutants described below and affected in the mitochondrial respiratory chain. This **Table 2** is completed with **Fig. 3** in which the position of mutations in mutants affected in subunits encoded by the mitochondrial genome are represented.

2.3 Mutants affected in complex I or in complexes I and III

Mutants deficient for complex I can be easily scored on the basis of their impaired growth in the dark ($dk^{+/-}$ phenotype) [45]. Indeed, contrary to complex III or complex IV mutants that do not grow in the dark because they lack two phosphorylation sites, complex I mutants are still able to grow in these conditions **(Fig. 4)**. However, their growth is significantly slower than wild type because they only retain two of the three phosphorylation sites that are operational when electron transfer proceeds through the respiratory chain. Their oxygen consumption, which is mildly reduced compared to wild type and insensitive to rotenone, a complex I inhibitor, occurs via complex II and alternative type II NAD(P)H dehydrogenases

such as Nda1. Whatever the type of mutants considered (mitochondrial or nuclear), this growth selection proved to be successful, as shown below.

Complex I mutants affected in subunits encoded by the mitochondrial genome were mostly obtained by random mutagenesis with intercalating dyes [46-48]. They are affected in *nd1 (dum20, dum25)*, *nd5* (*dum23*, *dum5*) or *nd6* (*dum17*) mitochondrial genes. In addition, mitochondrial transformation by biolistic device allowed obtaining mutants for the *nd4* gene (*Δnd4*, L157P ND4) [43, 44]. The basis for the isolation of such transformants relies on the fact that, as stated before, complex I mutants are able to grow in the dark, albeit slower than the wild-type strain. Mutants deleted for the left part of the mitochondrial genome including the *cob* gene (*e.g.*, *dum11* mutant) and that are unable to grow in the dark are used as recipient strains for mitochondrial transformation. The biolistic transformation was realized with a fragment of the mitochondrial genome covering the deletion and containing the mutated *nd4* gene. After a two-month selection in the dark, transformants were recovered: most of them harbored the wild-type mitochondrial genome but a few of them incorporated the alteration in the *nd4* gene [43, 44]. The possibility to perform site-directed mutagenesis in the *Chlamydomonas* mitochondrial genome is of particular interest since human mutations affecting mitochondria-encoded subunits of complex I cannot be reconstructed in the yeast *S. cerevisiae*. Therefore, *C. reinhardtii* represents an alternative model system as exemplified by the Leu_{157}Pro substitution introduced in the ND4 subunit of complex I in the L157PND4 mutant [44]. This substitution is present in the heteroplasmic state (mix of wild-type and mutant copies in mitochondria) in a patient presenting chronic progressive external ophthalmoplegia. When present in the homoplasmic state (only mutated copies in mitochondria) in *Chlamydomonas*, the mutation did not prevent the assembly of the 950 kDa whole complex I which conserves nearly all the NADH dehydrogenase activity of the peripheral arm. However, the NADH:duroquinone oxidoreductase activity was strongly reduced. Due to its nature, the introduced proline could disturb the organization of the transmembrane domain where the substitution is found and affect ubiquinone fixation to the membrane domain. The *in vitro* defects were correlated *in vivo* with decreased respiration and growth rates in heterotrophic conditions.

Seven complex I mutants affected in subunits encoded by the nuclear genome were isolated: four knock-down mutants corresponding to ND3, ND4L, ND7 and

ND9 subunits were obtained by RNA interference [39] and three knock-out mutants in the *NUOB10* (PDSW subunit), *NDUFS3* (ND9 subunit) and *NUOP4* genes by insertional mutagenesis [38].

Mitochondrial mutants affected in both complexes I and III could also be isolated by random mutagenesis. They are characterized by large deletions of the mitochondrial genome encompassing *cob* and *nd4* (*dum24*) or *cob*, *nd4* and *nd5* (*dum22*).

From the analysis of complex I assembly by Blue Native PAGE, we could propose a modular arrangement of the subunits which are concerned by the mutations, all highly hydrophobic and targeted to the inner mitochondrial membrane. While the wild-type complex I has a molecular weight of 950 kDa, the loss of ND4, ND5 or PDSW subunits leads to the assembly of a 700-kDa membrane-bound subcomplex. In contrast, the absence of intact ND1, ND3, ND4L or ND6 subunits totally prevents complex I assembly. This suggests that ND4, ND5 and PSDW, on one hand, and ND1, ND3, ND4L, and ND6, on the other hand, are located in two different membrane domains of the complex I membrane arm. This hypothesis is in good agreement with structural models proposed for the localization of these subunits within for example *Arabidopsis* [9] or bovine complex I [49].

2.4 Mutants affected in complexes III or IV and associated revertants

Mutants affected in complex III or complex IV can be easily scored on the basis of their lack of growth in the dark (dk^- phenotype, see above). These dk^- mutants can also be identified when cultivated in the light, using an *in vivo* staining test directly performed on Petri dishes. In contrast to wild-type colonies which reduce 2,3,5 triphenyltetrazolium chloride (TTC) to Red Formazan and become purple, mutant colonies deprived of complex III or complex IV remain green when Petri dishes are incubated in the dark with TTC [50]. Mutants affected in complex III or complex IV are thus obligate photoautotrophs that are deprived of the cytochrome pathway of respiration. Their oxygen consumption, which is insensitive to cyanide, is reduced and occurs via the activity of the SHAM-sensitive alternative oxidase.

Mitochondrial mutants affected in complex III harbor mutation in the *cob* gene and those affected in complex IV harbor mutation in the *cox1* gene [46]. Many of the

mutants deprived of complex III have a terminal deletion of the left end of the mitochondrial genome including the *cob* gene (*e.g.*, *dum1* and *dum11*), leading to the absence of complex III activity. The deletion mutants exhibit complex mitochondrial genomes, deleted monomeric genomes always coexisting with dimers resulting from head-to-head fusions between deleted monomers. In addition, one point mutation affecting the *cob* gene has also been identified (*dum15*), consisting in a two base-pair substitution that transforms the TCT codon (Ser) into TAC codon (Tyr) at position 140. Mutants resistant to myxothiazol (and mucidin) have also been isolated: they show an A to T nucleotide substitution in the *cob* gene, leading to a change of a Phe codon to a Leu codon at position 129 (*MUD2* mutant). Two mutants affected in *cox1* (*dum18* and *dum19*) present frameshift mutations (deletion or addition of T in runs of Ts) in *cox1* and are deprived of complex IV activity and assembly.

In the course of the culturing of these frameshift mutants, revertants were isolated. A revertant of the *dum18* mutant (presenting a + 1 T addition in a run of four Ts, located at codon 145 of the mitochondrial *cox1* gene) was characterized. In addition to the + 1 T frameshift mutation still present at codon 145, an A to C nucleotide substitution was found at codon 146, leading to the replacement of a Glu amino acid by an Ala amino acid in the polypeptide chain. No other mutations were detected in the *cox1* coding sequence. As the new GCG codon (Ala) created at position 146 is very seldom used in the mitochondrial genome of *C. reinhardtii*, it was suggested that the partial frameshift suppression by the nearby substitution was due to an occasional abnormal translocation of the ribosome (+ 1 base shift) facilitated both by the run of Ts and the low level or weak interaction of alanyl-tRNA [51].

Similarly, a revertant of the *dum19* mutant (presenting a -1T deletion in a run of 3 Ts at codon 152) was characterized. A genetic and molecular analysis demonstrated that the revertant phenotype is the consequence of two additional mutations that together act as a frameshift suppressor: an *m* mutation affecting a mitochondrial gene other than *cox1* and an *n* mutation affecting an unknown nuclear gene. Sequencing analysis showed that the *m* mutation affects the GTPase-associated domain of the large subunit (LSU) of mitochondrial rRNA. To our knowledge, this was the first example of a mutation in the GTPase-associated domain acting as a suppressor of a frameshift mutation [52]. In order to analyze the impact of the *m* mutation on the mitochondrial translational machinery, a strain carrying the *m*

mutation but wild-type for the *cox1* gene was isolated. The growth and the respiratory rate of the *m* mutant were affected and the activities of complexes I, III, and IV, all containing mitochondria-encoded subunits, were lowered. In contrast the activities of complex II and of the alternative oxidase, both encoded exclusively by the nuclear genome, were not modified. The steady-state levels of complex I enzyme and of several components of the respiratory complexes I, III, and IV were also reduced in the mutant [53].

Nuclear mutants affected in the *COX3* and *COX17* genes of complex IV were isolated by RNA interference. The *COX3* gene encodes a core subunit of mitochondrial cytochrome *c* oxidase (complex IV) whereas the *COX17* gene encodes a chaperone delivering copper to the enzyme. The *COX3*-RNAi mutant behaved like a mitochondrial mutant affected in *cox1* and presented no activity and assembly of complex IV. Due to its reduced respiration, it produced less H_2O_2 in the dark. The *COX17*-RNAi mutant presented a reduced activity of the cytochrome *c* oxidase, no modification of respiration and of H_2O_2 production in the dark but a two to threefold increase of H_2O_2 in the light compared to wild type and the *COX3*-RNAi mutant. The *COX17*-RNAi mutant was more sensitive to cadmium than the wild-type and *COX3*-RNAi strains. This suggested that besides its role in complex IV assembly, Cox17 could have additional functions in the cell such as metal detoxification or Reactive Oxygen Species protection or signaling [40].

2.5 Mutants affected in complex V

The seventeen subunits that compose mitochondrial ATP synthase in *Chlamydomonas* are all nucleus-encoded. Nuclear mutants affected in the *ATP2* and *ASA7* genes were isolated by RNA interference. *ATP2* gene codes for the catalytic β subunit and in the corresponding *ATP2*-RNAi knock-down lines, complex V was not assembled, respiratory rate was decreased by half and ATP synthesis coupled to the respiratory activity was fully impaired [54]. Like complex III and IV mutants, the *ATP2*-RNAi knock-down mutant was an obligate photoautotroph. In addition, as observed in yeast mutants [55, 56] lack of ATP synthase in *Chlamydomonas* also affected the morphology of mitochondria, which were deprived of cristae. In contrast, the loss of Asa7 subunit had no impact on cell bioenergetics or mitochondrial structures [31]. In the *ASA7*-RNAi knock-down line, the loss of Asa7 rather

destabilizes the enzyme dimeric form *in vitro* and renders growth, respiration, and ATP level sensitive to oligomycin [31].

Oligomycin is a potent inhibitor of H^+ channeling through the F_O moiety of mitochondrial ATP synthase, with no or only a weak effect on chloroplast photophosphorylation (reviewed in [57]). Growth, respiration, and ATP levels in *Chlamydomonas* and other relative species are however barely affected by oligomycin concentrations that affect other eukaryotes species. These observations led us to propose that the recruitment of novel ASA polypeptides and the massive modification of complex V stator might have conferred novel properties, including the stabilization of the enzyme dimeric form and the shielding of the proton channel [31].

2.6 Mutants affected in alternative enzymes

A mutant defective for the alternative oxidase (Aox1) has been isolated by RNA interference [58]. This *AOX1*-RNAi knock-down mutant displays a doubling of the cell volume and biomass without alteration of the generation time or change in total respiratory rate, with a significantly higher ROS production. A comparative study of both the mitochondrial and the cellular soluble proteomes was undertaken and indicated a strong up-regulation of the ROS scavenging systems and important modifications of proteins involved in the primary metabolism, namely an increase of enzymes involved in anabolic pathways and a concomitant general down-regulation of enzymes of the main catabolic pathways [58].

A mutant defective for a mitochondrial type II NADH dehydrogenase (Nda1) has been isolated by RNA interference. The *NDA1*-RNAi knock-down mutant presents a very mild phenotype, with only a slight decrease of respiration and no growth defect in heterotrophic conditions. In contrast, a double mutant affected in both Nda1 and complex I displayed strong alteration of respiration and growth rates in heterotrophic conditions, suggesting that Nda1 plays a role in the oxidation of matrix NADH in the absence of complex I [34].

2.7 Mutants affected in the mitochondrial codon usage

Two mutants with modified mitochondrial codon usage for the GGG codon were obtained by mitochondrial transformation [59]. In *Chlamydomonas*, the

mitochondrial codon usage is highly biased [60]. Some codons are more used than others and for example the 4 codons coding for the Gly amino acid (GGA, GGC, GGU and GGG) are not used with the same frequency. The GGT and GGC codons represent 5.9% and 1.4% of the mitochondrial codon population respectively whereas the GGA and GGG codons only represent 0.4% and 0.1% respectively. In the two mutants obtained, 10 GGT/GGC codons (*e.g.* T11-10 mutant) and 11 GGT/GGC codons (*e.g.* T22-11 mutant) were modified in GGG codons in the 3' end of the *nd4* gene. Consequently, the percentage of GGG codons in the mitochondria of the two mutants increased from 0.1% in wild type to 0.42% in the T11-10 mutant and 0.45% in the T22-11 mutant. Northern blot analysis showed that *nd4* gene expression was not affected in the mutants. However, physiological analysis showed that they were altered in respiration and in growth rate. Biochemical analysis on the T22-11 revealed reduced respiratory enzymes activities and reduced amounts of complexes I and IV. Thus, the codon modification on the *nd4* gene not only affected the complex I that contains the ND4 protein but also affected the complex IV, a respiratory complex that contains a mitochondria-encoded subunit *i.e.* the COX1 protein. In contrast, complex V that did not bear any mitochondria-encoded subunit in *Chlamydomonas* was not affected. The general reduction of respiratory enzymes containing mitochondria-encoded subunits was explained by the decrease of mitochondrial translation detected by *in organello* protein synthesis. This effect was probably linked to the limitations of the pool of mitochondrial transfer RNAs (tRNA) that could not be adapted to the new needs of mitochondrial genome [59]. Indeed, the introduction of 11 GGG codons in the *nd4* gene probably broke the established codon-tRNA balance causing the decrease of translational efficiency in mitochondria [61].

2.2.6 Mutants affected in the transcription of the mitochondrial genome

One mutant affected in the transcription of the mitochondrial genome has been described. This mutant named *stm6* was obtained by random insertional mutagenesis and is affected in the nuclear *MOC1* gene [62]. The Moc1 protein belongs to the mitochondrial transcription Termination Factor (mTERF) family. The knowledge about the function of these mTERF proteins in photosynthetic organisms is scarce but in metazoans, these proteins interact with the mitochondrial DNA and regulate transcriptional initiation and termination. Moc1 is targeted to mitochondria and is

essential for light acclimation. The loss of Moc1 in the *stm6* mutant indeed causes a pleiotropic phenotype characterized by sensitivity to high light, perturbed transcription profile of the respiratory complexes as well as reduced amounts of complex IV and rotenone-insensitive NAD(P)H dehydrogenase in light-grown cultures. A more detailed study of Moc1 showed that the protein binds specifically to an octanucleotide motif within the mitochondrial rRNA-coding module S3 (**Fig. 3**) and acts as a transcription terminator by blocking the transcription read-through of the leftward transcription unit of the genome [63].

3. Conclusion

The isolation and characterization of mutants affected in respiratory genes is useful to decipher the role of the numerous subunits that compose the respiratory-chain complexes, with special emphasis on subunits that are typical or specific to *Chlamydomonas*, like the Asa subunits of complex V, or the role of enzymes which regulate electron flow, such as type II NAD(P)H dehydrogenases. In addition, random mutagenesis can lead to the discovery of new genes involved in the assembly of the different complexes. This is especially relevant for complex I where the number of chaperones identified (9) is low compared to that of the other respiratory complexes, *e.g.* complex IV where 28 assembly factors have been identified to date. In addition, the availability of mitochondrial transformation opens the way to reverse genetics and a better knowledge of the small mitochondrial genome of *Chlamydomonas*.

At last, the availability of mutants affected to various extents in their ability to couple ATP synthesis to NADH oxidation is also a useful tool to study the relationship between the mitochondrial energetic status of the cell, hydrogen production, carbon metabolism, and ATP/NADPH adjustment for photosynthesis [62, 64-67].

Table 1: Protein components of mitochondrial respiratory complexes and assembly factors in *Chlamydomonas*

The present table is an update of the data presented in [1]. For *C. reinhardtii* the Genbank accession number and the gene name are given. References for the new data are indicated. (a) [1], (b) [17], (c) [68], (d) [69], (e) [70], (f) [71], (g) [72], (h) [73], (i) [74], (j) [75], (k) [76], (l) [77], (m) [22], (n) [29], (o) [78], (p) [58], (q) [79], (r)[34]

Table 2: List of respiratory-deficient mutants of *Chlamydomonas.*

((a) [48], (b) [45], (c) [47], (d) [43], (e) [44], (f) [39], (g) [38], (h) [46], (i) [80], (j) [41], (k) [50], (l) [81], (m) [82], (n) [40], (o) [54], (p) [31], (q) [58], (r) [34], (s) [59], (t) [62], (u) (Massoz S., Larosa V., Lapaille M., Remacle C., and Cardol P., unpublished data)

Legends of figures

Fig. 1: Complex I and ATP synthase of *Chlamydomonas*

A. Characteristic L-like shape structure of plant complex I. In addition to the two functional domains responsible for proton translocation (membrane arm) and for NADH oxidation (soluble arm), plant complex I possesses a globular matrix-exposed extra module composed of the gamma-type carbonic anhydrase subunits (γ-CA) attached to the central part of membrane arm [13]. In *Chlamydomonas* mutants, two subcomplexes of 700 kDa and 250 kDa have been identified [47].

B. Schematic representation of the dimeric ATP synthase supercomplex in *Chlamydomonas*. In dark-grey is represented the complex V with its F_1 and F_O domains linked by the central stalk. In light-grey are represented the peripheral stalk and the dimerization module constituted of algal-specific subunits (ASA subunits) [29].

Fig. 2: Histogram showing the number of structural and assembly components for each complex in each organism.

Fig. 3: Physical map of the 15.8-kb mitochondrial genome of *C. reinhardtii*.

The rectangles represent protein-coding genes: *cob*, gene encoding apocytochrome *b* of complex III; *nd1*, *2*, *4*, *5*, and *6*, genes encoding the corresponding subunits of complex I; *cox1*, gene encoding the subunit 1 of complex IV, *rtl*: reverse transcriptase-like protein. L and S represent modules encoding segments of rRNA of the large and the small subunits, respectively. W, Q and M represent tRNAs for Tryptophane, Glutamine and Methionine, respectively. The inverted telomeric ends are represented by short arrows and the bidirectional origin of transcription between *nd5* and *cox1* is represented by longer arrows. Positions of the mitochondrial mutants listed in Table 2 are indicated: deleted fragments are indicated by tear lines, mutations are indicated by black spots and the region with modified Glycine codons is indicated with a hatched square.

Fig. 4: Growth of the wild type and the respiratory mutants in the light and in the dark.

The respiratory mutants can be divided into two phenotypic classes when cultivated under heterotrophic conditions. One class is composed of mutants of complex III and

complex IV which are unable to grow in the dark (dk^- phenotype) and are thus obligate photoautotrophs, and another class is composed of mutants of complex I which grow in the dark but much more slowly than the wild-type strain ($dark^{+/-}$ phenotype). WT corresponds to the wild type strain; CI- corresponds to a complex I mutant; CIII-/CIV- corresponds to a complex III or complex IV mutant.

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Tables

Table 1

H. sapiens/B. taurus	*N. crassa/Y. lipolytica*	*Arabidopsis thaliana*	*Chlamydomonas reinhardtii*	References
Complex I				
Bacterial core				
NDUFS7/PSST	NUO19.3/NUKM	At5g11770	XP_001700585, *NUO10*	a,b
NDUFS8/TYKY	NUO21.3c/NUIM	At1g16700, At1g79010	XP_001702368, *NUO8*	a,b
NDUFV2/24 kD	NUO24/NUHM	At4g02580	XP_001698508, *NUO5*	a,b
NDUFS3/30 kD	NUO30.4 (31)/NUGM	AtMg00070	XP_001690652, *NUO9*/*ND9*	a,b
NDUFS2/49 kD	NUO49/NUCM	AtMg00510	XP_001697607, *NUO7*/*ND7*	a,b
NDUFV1/51 kD	51/NUBM	At5g08530	XP_001702590, *NUO6*	a,b
NDUFS1/75 kD	NUO78/NUAM	At5g37510	XP_001692885, *NUOS1*	a,b
ND1	ND1/NU1M	AtMg00516	AAB93446, *nd1*	a,b
ND2	ND2/NU2M	AtMg00285	AAB93444, *nd2*	a,b
ND3	ND3/NU3M	AtMg00990	AAQ55461, *NUO3*/ND3	a,b
ND4	ND4/NU4M	AtMg00580	AAB93441, *nd4*	a,b
ND4L	ND4L/NULM	AtMg00650	AAO61142, *NUO11*/ND4L	a,b
ND5	ND5/NU5M	AtMg00513	AAB93442, *nd5*	a,b
ND6	ND6/NU6M	AtMg00270	AAB93445, *nd6*	a,b
Conserved surpernumerary				
NDUFA1/MWFE	NUO9.8/NIMM	At3g08610	XP_001698399, *NUOA1*	a,b
NDUFA2/B8	NUO10.5/NI8M	At5g47890	XP_001695875, *NUOB8*	a,b
NDUFB3/B12	NUO10.6/NB2M	At2g02510	XP_001700920, *NUOB12*	a,b
NDUFA5/B13	NUO29.9/NUFM	At5g52840	XP_001693453, *NUOB13*	a,b
NDUFS6/13 kD	NUO18.4/NUMM	At3g03070	XP_001703419, *NUOS6*	a,b
NDUFA6/B14	NUO14.8/NB4M	At3g12260	XP_001694042, *NUOB14*	a,b
NDUFA11/B14.7	NUO21.3b/NUJM	At2g42210	XP_001689829, *TIM17*	a,b
NDUFB11/ESSS	NUO11.7/NUWM	At3g57785, At2g42310	XP_001697702, *NUO17*	a,b
NDUFS5/PFFD	NUO11.5/NIPM	At3g62790, At2g47690	XP_001691060, *NUOS5*	a,b
NDUFB4/B15	NUO6.6/NUVM	At2g31490	XP_001693191, *NUOB4*	a,b
NDUFA12/B16.6	NUO14 (13.5)/NB6M	At1g04630, At2g33220	XP_001701450, *NUOB16*	a,b
DAP13/B17.2	NUO13.4/N7BM	At3g03100	XP_001699522, *NUO13*	a,b
NDUFB7/B18	NB8M	At2g02050	XP_001698082, *NUOB18*	a,b
NDUFS4/AQDQ	NUO21/NUYM	At5g67590	XP_001695601, *NUOS4*	a,b
NDUFA8/PGIV	NUO20.8/NUPM	At5g18800, At3g06310	XP_001700114, *NUOA8*	a,b
NDUFB9/B22	NI2M	At4g34700	XP_001698797, *NUOB22*	a,b
NDUFB10/PDSW	NUO12.3/NIDM	At1g49140, At3g18410	XP_001694041, *NUOB10*	a,b
NDUFA9/39 kD	NUO40/NUEM	At2g20360	XP_001702653, *NUOA9*	a,b
NDUFB8/ASHI	NUO20.1/NIAM	At5g47570	XP_001700273, *TEF29*	a,b
NDUFB2/AGGG	NCU01436	At1g76200	-	a,b
NDUFB1/MNLL	NUO20.9/NUXM	At4g16450	XP_001696533, *NUO21*	a,b
NDUFC2/B14.5B	NUO10.4	At4g20150 (NDU9)	XP_001693474, *NUOP1*	a,b
NDUFC1/KFYI	NCU08300/NUUM	At4g00585	XP_001697243	a,b
NDUFA3/B9	NUO9.5/NI9M	At2g46540	XP_001692978	a,b
NDUFAB1/ACPM	SDAP	AAM6246	XP_001699275, *ACP1*	a,b
NDUFA7/B14.5A	NCU08930/NUZM	At5g08060	XP_001703194	a,b

NDUFA4/MLRQ	NCU02016	At3g29970	-	a,b
NDUFB5/SGDH	NUO17.8	At1g67785	-	a,b
NDUFA10/42 kD	-	AAG51141	-	a,b
XP_001253523	NCU03188	At3g47930 L-galactono-1,4-lactone dehydrogenase	XP_001693696, *GLDH*	a,b
Plant specific				
-	-	γ carbonic anhydrase At5g63510, At1g19580, At3g48680, At1g47260, At5g66510	XP_001703237, *CAG1*, XP_001701594, *CAG2*, XP_001696746, *CAG3*	a,b
-	-	At3g07480	XP_001699817, *NUOP3*	a,b
-	-	At5g14105	-	a,b
-	-	At1g67350	-	a,b
-	-	At1g68680	-	a,b
-	-	At2g27730	-	a,b
-	-	-	AAS58503, *NUOP5*	a,b
-	-	-	AAS58498, *NUOP4*	a,b
Assembly Factors				
NDUFAF1	CIA30	At1g17350	XP_001701850, *NUOAF1*	a,b
-	CIA84	-	-	a,b
NUBPL	IND1	At4g19540 (INDL)	XP_001702721	a,b
Foxred1	-	At2g24580, Sarcosine oxidase family protein	XP_001692123, FAD-dependent oxidoreductase	a,b
C8ORF38	-	At1g62730	XP_001693265	a,b
C20ORF7	-	At1g22800	XP_001693605	a,b
NDUFAF2 (B17.2L)	-	-	-	a,b
NDUFAF3	-	At3g60150	XP_001702394	a,b
NDUFAF4, (C6ORF66)	-	-	XP_001701912	a,b
H. sapiens*/*B. taurus	***Saccharomyces cerevisiae***	***Arabidopsis thaliana***	***Chlamydomonas reinhardtii***	
Complex II				
SDHA	SDH1	At2g18450, At5g66760	XP_001689842, *SDH1*	a
SDHB	SDH2	At5g40650, At3g27380	XP_001696290, *SDH2*	a
SDHC (QPS1)	SDH3	At5g09600	XP_001689507, *SDH3*	a
SDHD	SDH4	At2g46505	XP_001689952, *SDH4*	a
-	-	At1g47420	-	a
-	-	At1g08480	-	a
Assembly Factors				
-	TCM62	-	-	
SDHAF1	YDR379C-A	At2g39725	XP_001693672	c,d
-	FLX1	-	XP_001701258	c,e
SDH5	SDH5	At5g51040	XP_001694005	c,f
Complex III				
UQCRC1	COR1	At3g02090	XP_001697021, *QCR1*	a
UQCRC2	COR2	At3g16480, At1g51980	XP_001691043, *QCR2*	a
-	-	-	XP_001697130, *MPPA1*	a
CYB	COB	AtMg00220	AAB93440, *cob*	a
CYC1	CYT1	At3g27240, At5g40810	AAG44483, *CYC1*	a
UQCRCFS1	RIP1	At5g13440, At5g13430	XP_001689782, *RIP1*	a

UQCRQ	QCR7	At4g32470, At5g25450	XP_001696308, *QCR7*	a
UQCRB	QCR8	At3g10860, At5g05370	XP_001697451, *QCR8*	a
UQCRH	QCR6	At2g01090, At1g15120	XP_001697864, *QCR6*	a
UQCR10	QCR9	At3g52730	XP_001696682, *QCR9*	a
UQCR10	QCR10	At2g40765	XP_001699549, *QCR10*	a
UQCRFS1	-	-	-	a
Assembly Factors				
BCS1	BCS1	At5g17760	XP_001700670, *BCS1*	g
ABC1	ABC1	At4g01660	XP_001702520, *ABC1*	g
-	BCA1	-	-	g
CBP3	CBP3	At5g51220	XP_001689784	g
-	CBP4	-	-	g
-	CBP6	-	-	g
TTC19	-	-	-	g
-	CYC2	-	-	g
LYRM7/MZM1L	MZM1	-	XP_001690610	g,h
Cytochrome *c*				
CYC/CytC	CYC1	At1g22840, At4g10040	XP_001696912, *CYC*	a,g
Assembly factors				
Cytochrome c *maturation system*				
III	III	I	III	a,g,i
CCHL	CYT2 (CC1HL)	-	XP_001697002, *HCS1*	a,g,i
-	CYC2	-	-	a,g,i
-	CYC3	-	XP_001699246, *HCS2*	a,g,i
-	-	-	XP_001696883, *HCS3*	a,g,i
-	-	AtMg00830, AtMg00900, AtMg00960, AtMg00110, AtMg00180, At1g15220, At3g51790		a,g,i
Complex IV				
MTCOXI (COX1)	COX1	AtMg01360	AAB93443, *cox1*	a,j
MTCOXII (COX1)	COX2	AtMg00160	AAK30367, *COX2a*	a,j
-	-	-	AAK32117, *COX2b*	a,j
MTCOIII (COX3)	COX3	AtMg00730	AAG17279, *COX3*	a,j
COXVb (COX5b)	COX4	-	XP_001693699, *COX4/5b*	a,j
COXIV-1 (COX4-1)	COX5a	-	-	a,j
COXV-2 (COX4-2)	COX5b	At3g15640, At1g80230	-	a,j
-	-	At3g62400, At2g47380 COX5C	XP_001697444, *COX5c*	a,j
COXVa (COX5a)	COX6			a,j
COXVIIa (COX7a)	COX7	-	-	a,j
COXVIIc (COX7c)	COX8	-	-	a,j
COXVIc (COX6c)	COX9 (Cox7a)	-	-	a,j
COXVIIb(COX7b)	-	-	-	a,j
COXVIII (COX8)	-			a,j
COXVIb (COX6b)	COX12 (Cox9)	At1g22450, At4g28060	XP_001691399, *COX12*	a,j

COXVIa (COX6a)	COX13 (Cox10)	At4g37830	XP_001692867, *COX13*	a,j
-	-	-	AAM88388, *COX90*	a,j
Assembly Factors				
Membrane insertion and processing of the CIV subunits				
OXA1L, NP_005006	OXA1	At5g62050	XP_001693158 (partial), *OXA1*	a,j
COX20, NP_932342	COX20	-	-	a,j
COX18, AAI43643	COX18	-	ACM07437, *COX18*	a,j
-	MSS2	-	-	a,j
-	MSS51	-	-	a,j
-	PNT1	-	-	a,j
-	IMP1	-	XP_001698755, *IMP1*	a,j
IMMP2L	IMP2	At3g08980	XP_001689557, *IMP2*	a,j
-	SOM1	-	-	a,j
Copper metabolism and insertion into the CIV				
COX17	COX17	At1g53030	AAF82382, *COX17*	a,j
SCO1, SCO2	SCO1, SCO2	At3g08950, At4g39740	XP_001701493, *SCO1*	a,j
COX11	COX11	At1g02410	XP_001700235, *COX11*	a,j
COX19	COX19	At1g66590	XP_001698539, *COX19*	a,j
COX23	COX23	At1g02160	XP_001699418, *COX23*	a,j
PET191	PET191	At1g10865	XP_001700244, *PET191*	a,j
CMC1/ CMC2	CMC1/ CMC2	At2g07681 At2g07771	-	a,j
Heme A biosynthesis				
COX10	COX10	At2g44520	XP_001703217, *COX10*	a,j
COX15	COX15	At5g56090	XP_001701703 (partial), *COX15*	a,j
FDX2	YAH1	At4g05450 At4g21090	XP_001703155, *MFDX*	a,j,k
ADR	ARH1	-	XP_001693401, *ARH1*	a,j,k
Assembly				
-	PET100	At4g14615, At1g52821	-	a,j
SURF1	SHY1	At3g17910	XP_001701449, *SUR1*	a,j
-	COX14	-	-	a,j
-	COA1/2/3	-	-	a,j
-	COX25	-	-	a,j
-	CMC3	-	-	a,j
-	COA4	-	-	a,j
Unknown function		-	-	a,j
COX16	COX16	At4g14145	XP_001703532, *COX16*	a,j
CSRP2BP	PET117	-	-	a,j
Supercomplex III+IV assembly Factors				
HIG2A	RCF1 (YML030W)	-	-	l,m
-	RCF2 (YNR018W)	-	-	l,m
Complex V				
Fo subcomplex				
ATP6/A	ATPA (ATP6)	AtMg00410, AtMg01170	XP_001689492, *ATP6*	a
ATP5F1/B	ATPB (ATP4)	AtMg00640	-	a
ATP5G3/C	ATPC (ATP9)	AtMg01080, At2g07671	XP_001701531, *ATP9A* XP_001701500, *ATP9B*	a

ATP5H/D	ATPD (ATP7)	At3g52300	-	a
ATP5I/E	ATPE (ATP21)	At5g15320	-	a
ATP5J2/F	ATPF (ATP17)	At4g30010	-	a
ATP5L/G	ATPG (ATP20)	At2g19680	-	a
ATP5J/F6	ATPH (ATP14)	-	-	a
ATP8/A6L	ATP8	AtMg00480	-	a
ATP5O/OSCP	ATP5	At5g13450	XP_001695985, *ATP5*	a
ATPI/IF1	INH1, STF1	At5g04750	-	a
-	STF2	-	-	a
-	ATPJ/I (ATP18)	-	-	a
F_1 subcomplex				
ATP5A1/α	α (ATP1)	AtMg01190, At2g07698	XP_001699641, *ATP1*	a
ATP5B/β	β (ATP2)	At5g08670, At5g08680, At5g08690	XP_001691632, *ATP2*	a
ATP5C1/γ	γ (ATP3)	At2g33040	XP_001700627, *ATP3*	a
ATP5D/δ	δ (ATP16)	At5g47030	XP_001698736, *ATP16*	a
ATP5E/ε	ε (ATP15)	At1g51650	XP_001702609, *ATP15*	a
-	ATPK (ATP19)	-	-	a
-	-	At2g21870 (ATP7, F_Ad)	-	a
-	-	-	XP_001692395, *ASA1* (MASAP) XP_001696742, *ASA2* XP_001700079, *ASA3* XP_001693576, *ASA4* XP_001697115, *ASA5* XP_001701878, *ASA6* XP_001696750, *ASA7* XP_001695222, *ASA8* XM_001694550, *ASA9*	a,n
Assembly Factors				
F_0 subcomplex				
-	ATP10	AAF18252	-	a,o
NP_150592	ATP23	At3g03420.1	XP_001691633	a,o
-	ATP25	-	-	a,o
OXA1L	OXA1	At5g62050	XP_001693158 (partial), *OXA1*	a,o
F_1 subcomplex				
ATPAF1	ATP11	At2g34050	XP_001690396, *ATP11*	a,o
ATPAF2	ATP12	At5g40660	XP_001697254, *ATP12*	a,o
-	FMC1	-	-	a,o
Alternative Oxidase Family				
-	AOX -AAC37481 (*N. crassa*)	At3g22370 (AOX1a), At3g22360 (AOX1b), At3g27620 (AOX1c), At1g32350 (AOX1d), At5g64210 (AOX2)	XP_001694605, *AOX1* XP_001703329, *AOX2*	a, p, q
Type-II NAD(P)H Dehydrogenase Family				
-	NDAe1, NDAe2, NDAi1	At1g07180 (NDA1), At2g29990 (NDA2), At4g28220 (NDB1), At4g05020 (NDB2), At4g21490 (NDB3), At2g20800 (NDB4), At5g08740 (NDC1)	XP_001698901, *NDA1* XP_001691969, *NDA5* XP_001703055, *NDA6* XP_001703056, *NDA7*	a,r

Table 1: Protein components of mitochondria respiratory complexes and assembly factors in *Chlamydomonas*

Table 2

Biochemical defect	Mutant name	Growth in the dark	Gene mutation	Obtained by	References
Complex I	*dum5*	+/-	1T deletion in the 3' UTR of the mitochondrial *nd5* gene	acriflavine treatment	a
	dum17	+/-	-1T at codon 143 of the mitochondrial *nd6* gene	acriflavine treatment	a
	dum20	+/-	-1T at codon 243 of the mitochondrial *nd1* gene	acriflavine treatment	b
	dum23	+/-	-1T at codon 145-146 of the mitochondrial *nd5* gene	acriflavine treatment	c
	dum25	+/-	deletion of two of the codons 199–203 of the mitochondrial *nd1* gene	acriflavine treatment	b
	Δnd4	+/-	deletion of codons 2-24 of the mitochondrial *nd4* gene	mitochondrial transformation with *dum11* strain	d
	L157P ND4	+/-	introduction of a Leu157Pro substitution in the mitochondrial *nd4* gene	mitochondrial transformation with *dum11* and *dum22* strains	e
	ND3-RNAi	+/-	no transcript detected for the nuclear *NUO3* gene	RNAi	f
	ND4L-RNAi	+/-	reduction of the transcript steady-state levels (98%) of the nuclear *NUO11* gene	RNAi	f
	ND7-RNAi	+/-	reduction of the transcript steady-state levels (95%) of the nuclear *NUO7* gene	RNAi	u
	ND9-RNAi	+/-	no transcript detected for the nuclear *NUO9* gene	RNAi	u
	amc 5/7	+/-	inactivation of the nuclear *NUOB10* gene	insertional mutagenesis	g
	amc14	+/-	partial inactivation of the of the nuclear *NUO9* gene expression	insertional mutagenesis	u
	amc15	+/-	partial inactivation of the nuclear *NUOP4* gene expression	insertional mutagenesis	u
Complex I and III	*dum22*	-	4.35 kb mitochondrial deletion encompassing the left telomere, *cob*, *nd4* and 3' end of *nd5*	acriflavine treatment	h
	dum24	-	3.5 kb mitochondrial deletion encompassing the left telomere, *cob* and 3' end of *nd4*	acriflavine treatment	i
Complex III	*dum1*	-	1.7 kb mitochondrial deletion, encompassing the left telomere and the entire *cob* gene	Acriflavine treatment	j
	dum11	-	1.2 kb mitochondrial deletion encompassing the left telomere and the end of *cob* gene	ethidium bromide treatment	k
	dum15		introduction of a Ser140Tyr substitution in the mitochondrial *cob* gene	ethidium bromide treatment	k,l
	MUD2		introduction of a Phe129Pro substitution in the mitochondrial *cob* gene	selection on mucidin	m
Complex IV	*dum18*	-	+1T at codon 145 of mitochondrial *cox1* gene	Acriflavine treatment	l
	dum19	-	-1T at codon 152 of mitochondrial *cox1* gene	Acriflavine treatment	l
	COX3-RNAi	-	no transcript detected for the nuclear *COX3* gene	RNAi	n
	COX17-	+	no transcript detected for the	RNAi	n

	RNAi		nuclear *COX17* gene after treatment with copper or cadmium		
Complex V	ATP2-RNAi	-	lack of the β subunit protein as a result of the silencing of the nuclear *ATP2* gene	RNAi	o
	ASA7-RNAi	+	lack of the Asa7 subunit protein as a result of the silencing of the nuclear *ASA7* gene	RNAi	p
SHAM-sensitive pathway of respiration	AOX-RNAi	+	lack of the Aox1 protein as a result of the silencing of the nuclear *AOX1* gene	RNAi	q
	NDA1-RNAi	+	lack of the Nda1 protein as a result of the silencing of the nuclear *NDA1* gene	RNAi	r
Complex I and IV	T11-10	+/-	replacement of the 10 GGT/GGC codons by GGG codons in the 3' end of mitochondrial *nd4* gene	mitochondrial transformation with *dum11* strain	s
	T22-11	+/-	replacement of the 11 GGT/GGC codons by GGG codons in the 3' end of mitochondrial *nd4* gene	mitochondrial transformation with *dum22* strain	s
Mitochondrial transcription profile	*stm6*	+/-	inactivation of the nuclear *MOC1* gene	insertional mutagenesis	t

Table 2: List of respiratory-deficient mutants of *Chlamydomonas*

Figure 1

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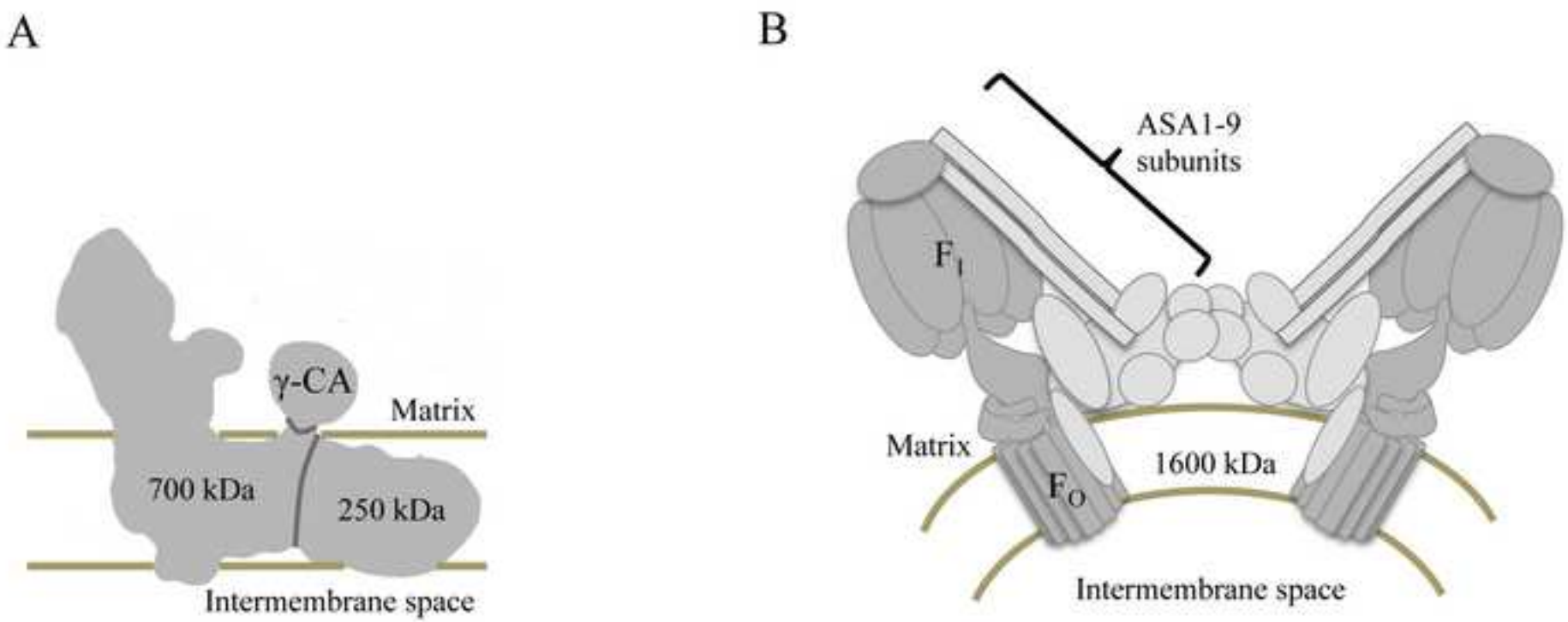

Figure 2

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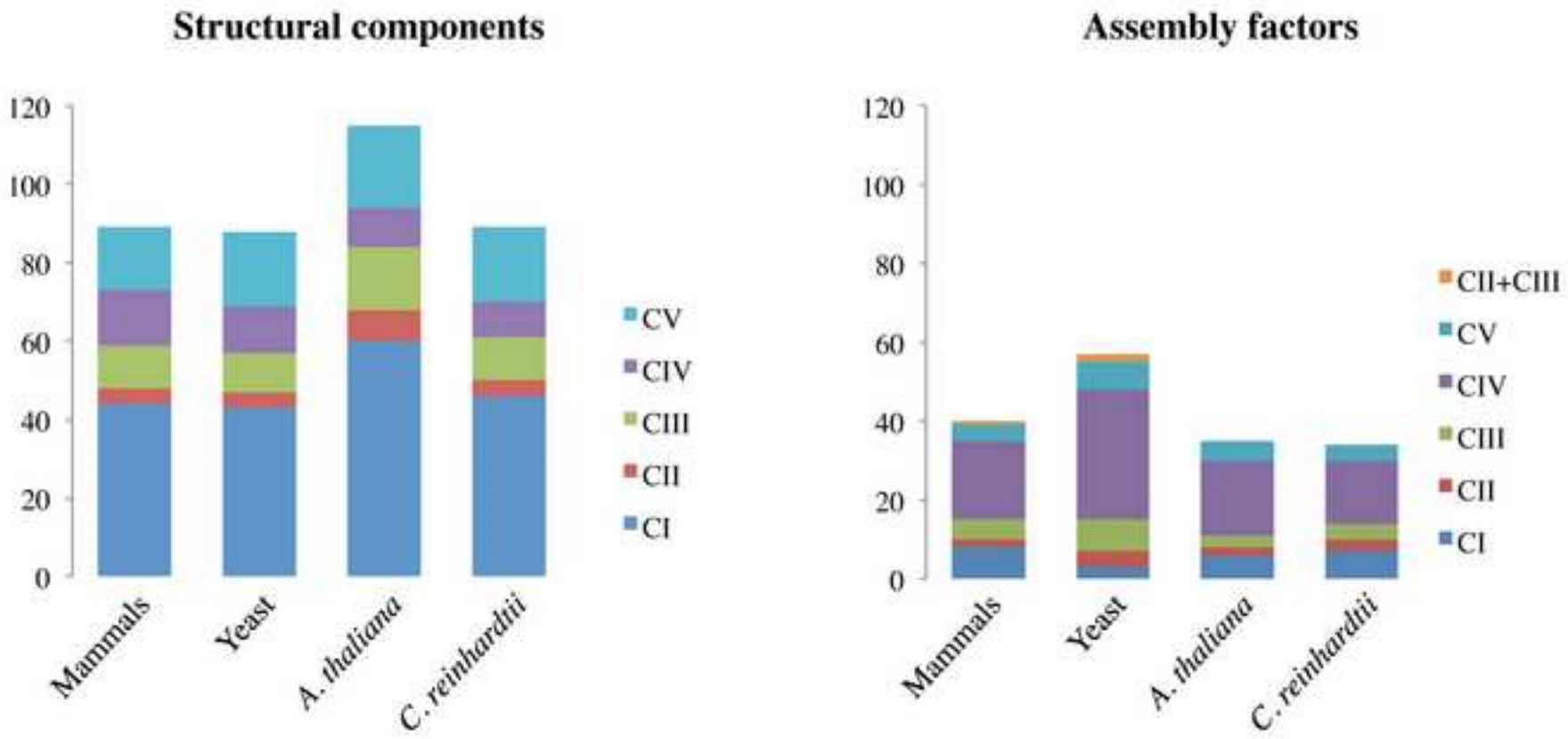

Figure 3

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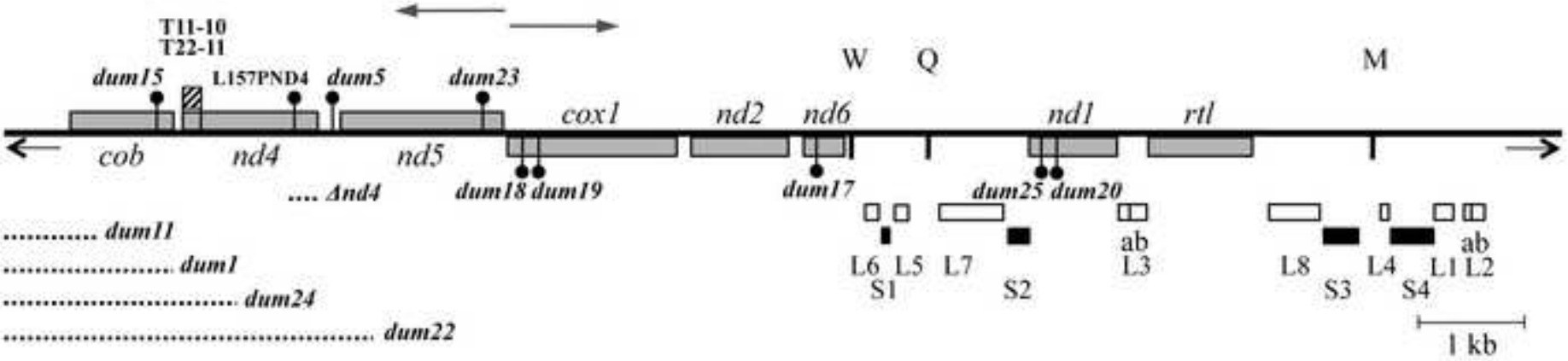

Figure 4

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